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Comparisons of responses by planarian to micromolar to attomolar dosages of morphine or naloxone and/or weak pulsed magnetic fields: Revealing receptor subtype affinities and non-specific effects

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Abstract

Purpose: **The behavioral responses of planaria to the exposures of a range of concentrations of morphine (μM to attoM) or the μ-opiate antagonist naloxone or to either of these compounds and a burst-firing magnetic field (5 μT) were studied.**

Material and methods: **The locomotor velocity (LMV) of planaria was measured after individual worms were exposed to increasing concentrations from attomolar to micromolar of morphine or naloxone, physiologically patterned magnetic fields or a combination of the two.**

Results: **Compared to spring water controls, the 2-hour exposure to the patterned magnetic field before measurement reduced activity by about 50% which was comparable to the non-specific effects of morphine and naloxone across all dosages except 1 attomolar that did not differ from spring water. The specific dosage of 100 nM produced additional marked reduction in activity for planaria exposed to either morphine or naloxone while only 1 pM of morphine produced this effect.**

Conclusion: **The results support the presence of at least two receptor subtypes that mediate the diminished activity effects elicited by morphine specifically and suggests that exposure to the specifically patterned magnetic field produces a behavioral suppression whose magnitude is similar to the 'dose independent' effects from this opiate.**

Keywords: Planaria, weak analgesic magnetic fields, morphine, naloxone, receptor subtypes

Introduction

Nociception and its avoidance are fundamental to the survival of organisms. Several opiate receptor subtypes each with a different affinity (effective concentration) mediate the effects of morphine within several species. Although there are multiple in vitro methods for discerning among these receptor subtypes, we have been pursuing behavioral indicators in aquatic animals, such as planarian, to discern the presence of these receptors by measuring their quantitative responses to a broad range (micro- to attomolar) of concentrations of ligand. Easily measured altered behaviors within narrow ranges of concentration allow relatively inexpensive and quick inferences of receptor subtypes. The possibility that the efficacy of agonists and antagonists, as well as exposure to 'physiologically' patterned electromagnetic fields, might involve similar receptors can also be discerned. Here we present evidence of at least two receptor subtypes for morphine displayed by planarian and the likelihood that particularly patterned weak magnetic field that is known to produce analgesia in vertebrates (Baker-Price and Persinger 2003, Martin et al. 2004, Martin and Persinger 2004) and invertebrates (Thomas et al. 1997) may involve one of these receptors as well as non-specific effects.

The development by Raffa et al. (2003) of a sensitive and convenient metric for planarian locomotor velocity (pLMV) to assess the responses of these organisms, in terms of gridlines crosses per unit time, has facilitated discernment of subtle changes to gradations of pharmacological substances. This measure was sensitive to dose-related exposures to cocaine (10^{-9} M to 10^{-5} M) and differential responses to κ-opioid receptor antagonists (Raffa et al. 2003). Umeda et al. (2004) showed that the decrease in locomotor velocity (LMV) associated with withdrawal from cocaine and κ-opioid compounds could be blocked by D-glucose but not by L-glucose. There are other methods of assessing planarian responses, such as the qualitative changes in motor behavior in response to opioid-dopamine interactions (Passarelli et al. 1999). We selected LMV because of the precision and marked reproducibility. The quantitative values for LMV for our untreated planarian, employing our samples sizes, were not significantly different than those reported by Raffa et al. (2003).

The isolation of the convergent operations that would allow mutual equivalence of the spatial organizations of matter as displayed by chemicals and the temporal organizations of information as displayed by temporally patterned electromagnetic fields could be as significant as

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the discovery of the Rosetta stone for the equivalence codes for archaeological languages. Several authors have pursued this potential relationship for the domain of nociception because of its importance in adaptation. In a seminal series of studies, Thomas et al. (1997) examined the effects of a pulsed magnetic field upon analgesic responses of the land snail as inferred by the pre-injection of either agonists or antagonists for μ, δ, or κ opioid receptors. They found that the specific pulsed magnetic fields elicited significant analgesic effects through mechanisms that involved δ and to less extent μ receptor subtypes. The results were anticipated by Kavaliers and Ossenkopp (1991) and Prato et al. (1995) who suggested a resonance-like dependence of the magnetic field effect.

Planarian's responses to different types of magnetic fields have been well documented. The classical work of Brown (1962) and Brown and Chow (1973) established that the orientation and movement directions of planarian could be modified experimentally by applying weak static magnetic fields (less than 10 times the intensity of the earth's magnetic field) in the horizontal plane. Recently Mulligan et al. (2012), employing an 'energy' resonance approach, showed that the movement of planarian was specific to weak, amplitude-modulated 7 Hz magnetic fields presented for only 6 min once per hour during the dark phase. The effect was enhanced with a specific concentration, based upon the predictions from the resonant model, of melatonin in the water medium. There are several researchers who have shown fission rates and regeneration of planarian respond in a non-linear manner to static fields between 10 and 3000 nT (Novikov et al. 2007, 2008) and to specific resonance-related frequencies within the 10–40 μT range. Such features may be analogous to differential sensitivities of receptor subtypes. The results also strongly support the role of calcium resonance (Jenrow et al. 1995, Titras et al. 1996, Reuter and Kreschenko 2004). Congruent changes in biomolecular pathways such as ERK (extracellular regulated signal kinases) and heat shock proteins (hsp70) levels have been replicated (Goodman et al. 2009, Madkan et al. 2009).

Planaria contain all of the major classical neurotransmitters, such as serotonin, acetylcholine, the catecholamines, gamma-amino butyric acid, and excitatory amino acids, that are present in mammals (Buttarelli et al. 2008). Synthesis of anandamide and expression of cannabinoid receptors as well as the localization of Met-enkephalin in the neutrophil of flatworms has been reported (Passarelli et al. 1999). The general consensus has been that the receptor for this enkephalin is the planarian equivalent of the mammalian κ-opioid receptor (Raffa et al. 2006). Because planaria lack a blood-brain barrier and their responses are so stereotyped, discernment of complex interactions should be less affected by the intrinsic impedances encountered with mammalian systems yet reveal potentially applicable information.

Materials and methods

Planarian maintenance and experimental procedures

Brown planaria (*Dugesia tigrina*) were obtained from Carolina Biological Supply (Burlington, NC, USA). The planaria were acclimated to local laboratory conditions (21°C), housed in darkness, and utilized in experiments within 72 h of arrival. Morphine sulphate solution and naloxone hydrochloride were obtained from Sigma-Aldrich Canada Ltd (Oakville, Ontario, Canada) and diluted in spring water to the desired molarity. Spring water was obtained from Feversham, Grey County, Ontario, Canada. Ion contents of the water in ppm were HCO3 270, Ca 71, Mg 25, SO4 5.9, Cl 2.7, NO3 2.6 and Na 1.

Worms were removed from group containers and placed in 1.5 ml plastic conical centrifuge tubes (Fisherbrand (Ottawa, Ontario, Canada) with flat-top snap cap with dimensions of 10.8×40.6 mm) composed of polypropylene. There was one worm per tube containing 1 ml of spring water. Each tube was held upright by placing it in a plastic rack that held 27 tubes per experiment. Consequently there were 27 planarian (27 tubes) per exposure or experiment. Within 1 min of being removed from group containers and placed in the plastic tubes, the worms were exposed for 2 h to a magnetic field or sham condition (no field) within a Helmholtz coil. The coil was $32.5 \times 32.5 \times 41$ cm long and was wrapped with 305 m of 30 (AWG) gauge wire (30 ohm), as described by Martin et al. 2004. The tube container was placed in the middle of the coil.

Pulsed magnetic field exposures

For the first part of the study, two patterns of magnetic fields whose analgesic effectiveness has been demonstrated in several studies for rats (Martin et al. 2004), human patients (Richards et al. 1993, Baker-Price and Persinger 2003), and invertebrates (Thomas et al. 1997), were tested. The two patterns (Figure 1) are frequency modulated fields generated from converting numbers between 0 and 256 to between

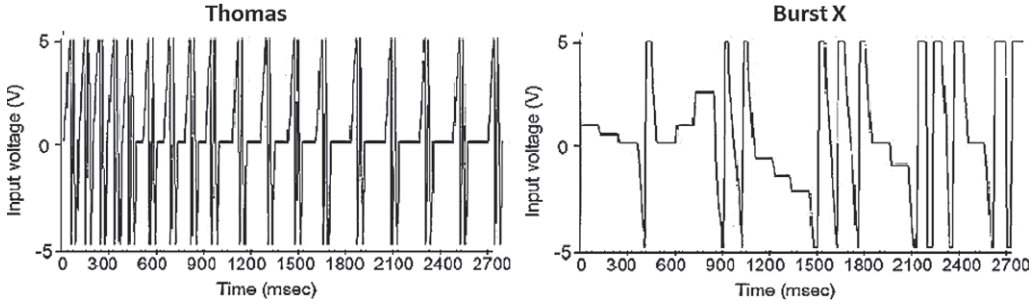

Figure 1. The two patterns of magnetic fields initially examined to compare the efficacy of their effects upon planaria activity when exposed to μM to attoM concentrations of morphine or naloxone.

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− 5 V and + 5 V with 127 as 0 V. The total numbers of points (values between 0 and 256) for the two patterned fields were 849 for the 'Thomas pulse' and 248 for the 'burst-x' pattern. The former was developed by Thomas and Persinger (1997) based upon theoretical and chirp-features of communication systems while the later was extracted simulation of burst-firing from averaged potentials from a human amygdala during the display of complex partial epileptic seizures (Richards et al. 1993).

The point duration, the time each incremental voltage determined by the number between 0 and 256, for both fields was 3 ms. This has been shown to be more effective to produce analgesic effects in rats than point durations that are more or less than that value (Martin et al. 2004). A similar effect was noted for related temporal patterns of magnetic fields for more protracted exposures upon mortality of planarian (Murugan et al. 2013). The time between the patterns was 3 ms for the Thomas pulse and 3000 ms for the burst-firing pattern in order to simulate the optimal conditions described for hotplate analgesia for rodents (Martin et al. 2004). The average (root mean square) of the field strength within the center of the coil where the 27 tubes were located was 5 μT (± 0.5 μT). A total of 70 worms were measured.

Behavioral testing

After this period, each worm was placed into a clear glass dish, containing spring water in order to measure velocity of movement. This procedure was similar to that first described by Raffa et al. (2003). The dish was placed over graph paper with gridlines separated by 5 mm. Each worm was placed in the center of the dish and the velocity was defined as the numbers of gridlines cross during a 5 min interval and expressed as the numbers of gridlines crossed per minute. The cumulative number was calculated as the measure of LMV. A given planarian was a subject only once. After each testing, the dish was rinsed with spring water to remove possible contamination of residual compounds.

Pharmacological compounds

In the second part of the study, the two test compounds (morphine, $n = 171$ planarian, and naloxone, $n = 156$ planarian) were diluted into aliquots of spring water with between 25×10^{-6} M to 10^{-18} M for morphine and 10^{-6} M to 10^{-15} M for naloxone. We selected this maximum concentration for morphine because 1 and 10 mM produced planarian mortality. Spring water was the 'control' or reference condition. The serial dilutions for the two drugs were completed by extracting 0.01 μl of solution with a digital micropipette and adding it to 15 ml of spring water. There were three worms per dilution for the nine dilutions during a given exposure and at least three replicates (different days) were completed. Each worm was placed individually into a conical centrifuge tube containing 1 ml of test solution and allowed to habituate for 2 h prior to tests for locomotor activity.

Treatment interactions and statistical analysis

In the third part of the study, based upon the results of part 1, groups of planaria were exposed to different concentrations of the test compounds (morphine, $n = 91$; naloxone, $n = 91$) while they were being exposed to the burst-x firing magnetic fields for 2 h. The primary statistical procedures involved multiple level analyses of variance, one-way analyses of variance with Scheffee's post hoc tests to differentiate the groups. All analyses involved SPSS 16 PC software.

Results

Pulsed magnetic field exposures

As shown in Figure 2, the planarian exposed for 2 h to either of the two temporally-patterned magnetic fields displayed significantly less movement during the 5 min after being removed from those conditions than did sham field-exposed planarian. The planarian exposed to the 'burst-x' pattern displayed significantly less movement than those exposed to the other field pattern. In fact, the activity was about one-half of the planarian exposed to the sham treatment. One-way analysis of variance demonstrated a significant difference,

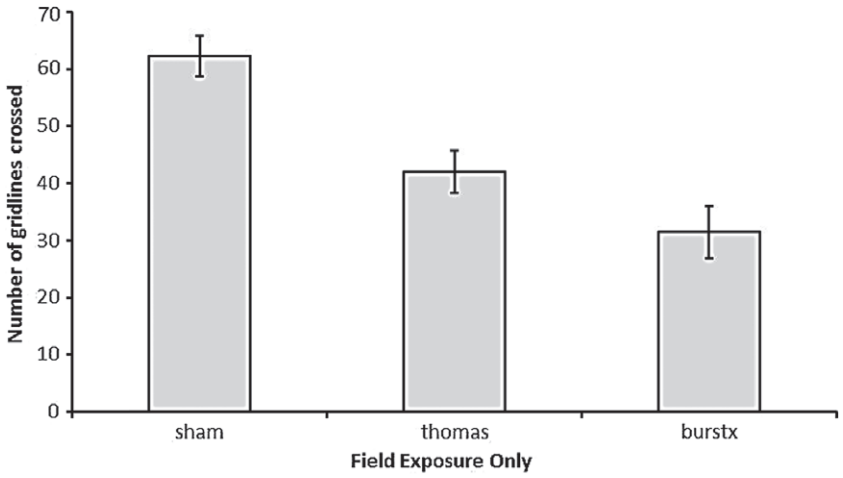

Figure 2. Mean total numbers of grid lines crossed (locomotor velocity or LMV) over 5 min for planarian after exposure for 2 hours to sham fields or to either the 'Thomas' pulse frequency-modulated or 'burst-x' magnetic fields with strengths around 5 microTesla. Vertical bars indicate standard errors of the mean (SEM).

indicated by the F-statistic (F), confidence interval (p), and measure of effect size (Ω^2), [$F(2,67) = 325.63$, $p < 0.001$; $\Omega^2 = 0.91$]. Post hoc analyses indicated that the worms exposed to the burst-x pattern field displayed lower LMV then those exposed to the Thomas pulse; however, the LMV of groups exposed to either of these fields were significantly lower than the sham-field exposed groups. In sham-field conditions, the tubes containing the worms were placed in the Helmholtz coil for the same duration as the field-exposed groups. However, no experimental fields were generated.

Drug exposures

The mean values for the LMV, as inferred by numbers of grids crossed, of planarian that were exposed within their aqueous environment to various concentrations of morphine between 25 μM and 10 attoM are shown in Figure 3. All dosages of morphine, except 10 attoM, produced suppressions of activity compared to the water only condition. Post hoc analyses of this very significant [$F(12,158) = 93.90$, $p < 0.001$; $\Omega^2 = 0.88$] difference in LMV as a function of dosage revealed that the 100 nM and 1 pM concentrations produced the greatest reduction in activity that was significantly lower than 10 fM, 1 uM, or 100 pM, which were in turn significantly lower than all of the other concentrations. There was no significant difference in LMV for the worms exposed to spring water or to 10 attoM of morphine.

Figure 4 shows the responses of the planarian to different concentrations of naloxone, the classic μ-opioid receptor antagonist. The planarian responded by diminished activity at the same concentrations 100 nM and 1 pM that produced this effect for morphine. Post hoc analyses of the significant [$F(11,144) = 33.43$, $p < 0.001$; $\Omega^2 = 0.71$] dosages differences indicated that the 100 nM and 1 pM dosages produced the lowest activity that was significantly different from all other dosages (except 1 fM and spring water) that did not differ significantly from each other. The spring water and 1 fM concentrations did not differ significantly from each other.

Combining drug and magnetic field exposures

Figure 5 shows the activity of the planarian when exposed to various concentrations of morphine and at the same time to the burst-x magnetic field pattern. Post hoc analyses indicated that the significant dosage differences [$F(8,72) = 15.40$, $p < 0.001$; $\Omega^2 = 63\%$] was due in large part to the lower LMV values for planarian exposed to the field + 100 nM and the field + 1 pM compared to all other concentrations. The 1fM + field and spring water conditions did not differ significantly from each other.

For comparison, the effects of simultaneous exposure to the burst-x magnetic field and various concentrations of naloxone are shown in Figure 6. The significant dose-differences [$F(8,72) = 19.49$, $p < 0.001$; $\Omega^2 = 0.68$] were due in large part to the lower activity for the field + 100 nm naloxone group which was significantly different from the field + 1 pM, 1 nM, 10 pM, and 10 μM groups who displayed less activity than all of the other groups that did not differ significantly (including water) from each other.

Clearly, the most conspicuous general effect of the exposure to this particular magnetic field configuration was the diminished activity, compared to sham-field controls, for all concentrations of drugs as well as for spring water. The planarian placed only in spring water averaged about 61 crossing while those exposed in spring water and the magnetic field traversed about 36 crossings (Figures 5 and 6), that is about 60% of control activity which was similar to the effects of all morphine concentrations except for 100 nM and 1 pM levels. On the other hand, those exposed in control conditions without any drugs displayed an

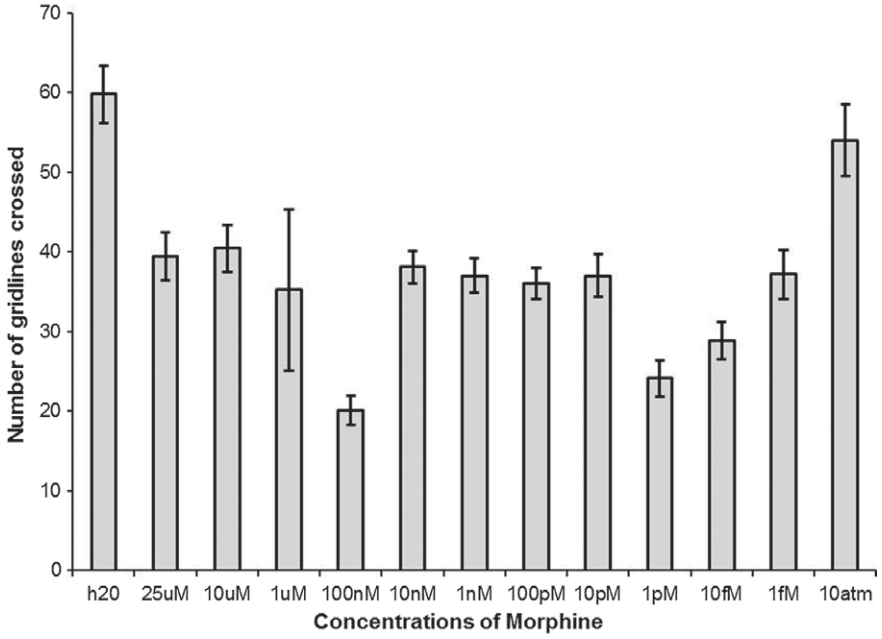

Figure 3. Mean total numbers of lines (LMV) crossed in 5 min for planarian exposed for 2 hours to different concentrations of morphine. Vertical lines indicated SEM. There was no statistically significant difference in activity between spring water (h20, vehicle control) only and morphine at 10 attomolar concentrations.

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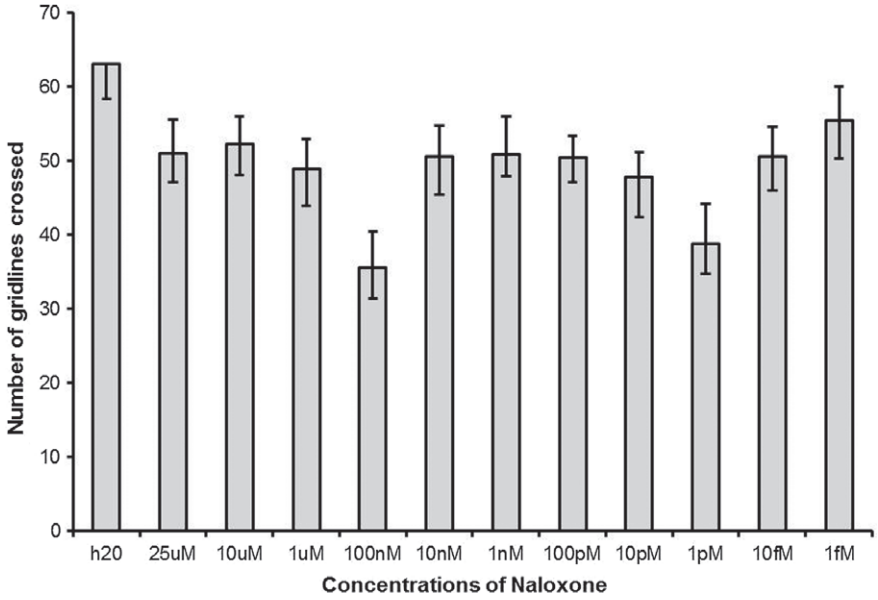

Figure 4. Mean total numbers of lines crossed in 5 min by planarian after being exposed for 2 hours to different concentrations of the opioid (μ) antagonist naloxone. Vertical bars are SEM. H_2O = vehicle control.

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average of 60 crossings compared to the 20 crossings produced by exposure to 100 nM of morphine, that is, about 33% of no-drug activity. Stated alternatively the diminished activity from the magnetic field was comparable to the 'non-specific' effect of the presence of morphine in the aqueous environment. The two exceptions to this effect were the greater diminished activity associated with the 100 nM and 1 pM concentrations.

To discern the specific effects, if any this magnetic field configuration exhibited upon the two putative 'receptors' dosages around the two enhanced concentrations, two-way analyses of variance were completed for the morphine and morphine + field conditions, and, for the naloxone and naloxone + field conditions for the concentrations that showed the greatest non-linear effect (diminishment) of activity: 1 μM, 100 nM and 10 nM and 10 pM, 1 pM, 10fM and 1 fM. The values below are reiterations, for convenience, of the means (with SEM) noted in the figures. The significant interaction [$F(2,65) = 4.67$, $p = 0.01$] between field + morphine and morphine was due to the significantly lower activity for the field + morphine (M = 28.7, 28.2) compared to the morphine only groups (M = 35.6, 37.5) for the 1 μM and 10 nM concentrations, respectively, while there was no difference for the 100 nM concentrations (19.5, 19.8). In other words, the field did not increase the diminished activity at the most efficacious dosage but widened the concentration range at which general reduction occurred. A similar two way interaction [$F(3,76) = 4.71$, $p < 0.01$] was noted for the lower concentrations. The LMV means for 10 pM, 1 pM, 10 fM and 1 fM for the morphine only and morphine + field treatments were 37.0, 24.4, 28.9 and 37.9; and 28.0, 21.1, 27.0 and 31.0, respectively.

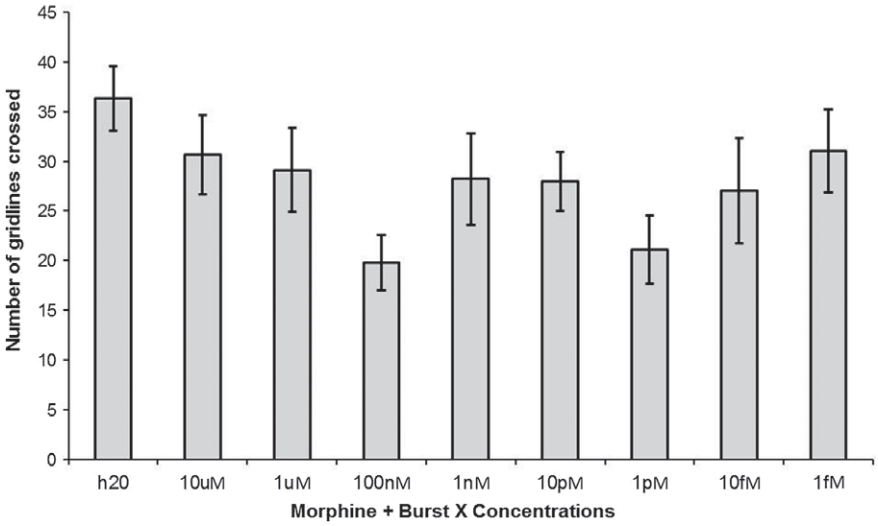

Figure 5. Planarian movements over 5 min following exposure for 2 hours to the burst-x magnetic field pattern and various dosages of morphine or water (h20). Vertical bars are SEM. H_2O = vehicle control.

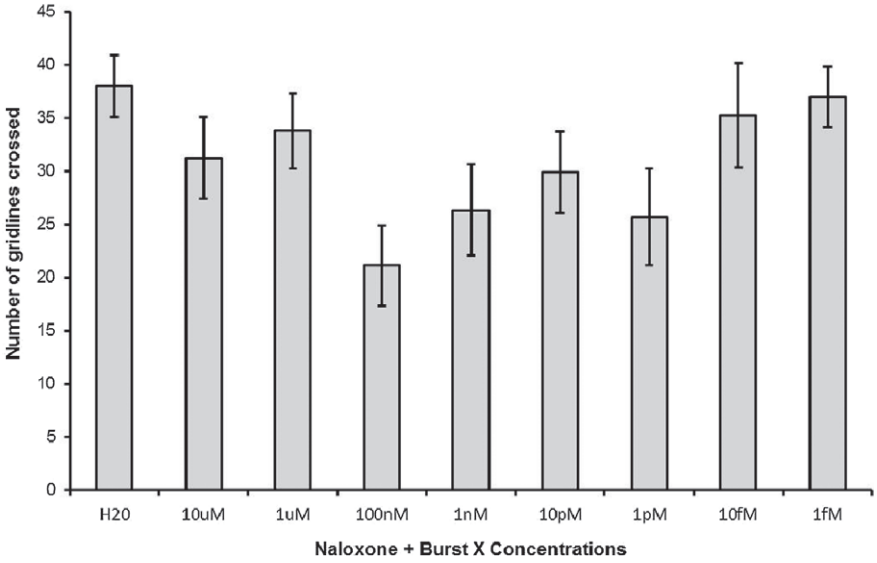

Figure 6. Mean total numbers of gridlines crossed in 5 min by planarian exposed for 2 hrs to the burst-x magnetic field and to various concentrations of naloxone. Compared to other concentrations and to spring water, the primary statistically significant diminishment was evident for the 100 nM concentrations. Vertical bars are SEM. H_2O = vehicle control.

The two-way interaction for the naloxone and naloxone + field treatments for these concentrations was strongly significant statistically [$F(2,60) = 10.63$, $p < 0.001$] and was due to even more diminished activity when both conditions were present for the 100 nM and 10 nM concentrations. The mean LMV for the 1 μM, 100 nM and 10 nM concentration groups exposed to the nalaxone or naloxone + field conditions were 48.8, 35.5, and 50.8, and, 45.1, 21.1, and 26, respectively. On the other hand there was no statistically significant interaction [$F(3,80) = 1.56$, $p > 0.05$] between field or sham conditions exposed to naloxone for these four concentrations within the pM range. Instead the activity of the planaria exposed to the field + naloxone were reduced across the concentrations compared to naloxone only [$F(1,80) = 268.27$, $p < 0.001$].

Discussion

The results of this study verify the sensitivity of Raffa's locomotor velocity (LMV) measures to discern potent changes in planarian behavior when exposed to remarkably low concentrations of opioid-related compounds in their aqueous environments. The mean value for the control planaria, those exposed to spring water only, in our study was 63 (Standard deviation [SD] = 4.6) while the value reported by Raffa et al. (2003) was 60 (SD = 3.5). It is clear that diminished activity can be induced in planaria at concentrations of morphine as low as 1 fM. At concentrations of 10^{-18} M the planaria respond as if they were moving in spring water.

These measurements also suggest there are at least two conspicuous receptors. This assumes their presence can be inferred by the enhancement of an agent's effect when it is administered at a specific concentration but not at lower or higher concentrations. This is consistent with other observations. The first putative receptor would have been most responsive to concentrations of morphine in the order of 100 nM while the second was most sensitive to concentrations of about 1 pM.

However by far the most important observation was the non-specific decrease in activity for planarian exposed to any concentration of morphine (that did not kill them) from 25 μM of morphine to 1 fM. A similar but less intense effect was noted for naloxone. This non-specific effect, which diminished the activity by 50% compared to control conditions, was the same magnitude as that produced by the patterned magnetic field only. These results strongly suggest that the multiple weak and strong responses of invertebrates to different opiate receptor subtype agonists and antagonists may reflect a major contribution from these non-specific effects. We have reasoned that appropriately patterned magnetic field effects are not mediated through receptor interaction or simulation. Instead, they are influencing a more fundamental process shared by all receptors within the plasma membrane.

One possible physical correlate of this process can be inferred by estimating the numbers of molecules required to produce the non-specific effect. The activity of planaria exposed to 10 attoM of morphine did not differ from those exposed to only spring water while those exposed to the next dosage of morphine (1 fM, 100 times greater concentration) showed the same magnitude of non-specific effects as μM dosages (25 billion times more concentrated). The estimated number of molecules within the 1 cc of 10^{-17} M (10 attoM) of solution removed from 15 cc of stock is ~ 4×10^5 whereas the numbers of molecules within the first effect dosage, 1 fM (4×10^7).

Assuming a planaria length of 6 mm, width of 0.5 mm and thickness of 0.05 mm, the volume would be 1.5×10^{17} nm^3. Consequently the volume of the planarian would be 1.5×10^{-4} of the volume of 10^{21} nm^3 (1 cc of fluid). Assuming homogeneous distribution of the morphine molecules from Brownian movement, this would mean that ~ 60 molecules of morphine inside the volume of a planarian would not be effective (no different than ambient spring water) to alter activity while ~ 6,000 molecules would diminish LMV as

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effectively as billions of molecules. If only brain volume was involved (~ 10^{16} nm^3, Okamoto et al. 2005) the number of effective molecules would be less by a factor of about 10. Simplistically, this suggests a mechanism involving relatively small numbers of channels or sequestering-points that once inactivated by physical blockage or chemical linkage reduces the activity of planarian by about 50%. Response specificity, that is preferential binding to proteins, would be superimposed upon this non-specific effect.

There are several theoretical and empirical reasons to suggest that the ion most associated with the non-specific effect involves H^+. Its influence could occur through the proton (water) channel (Decoursey 2003) within the cell membrane. Outward H^+ flux is important for reducing intracellular shifts from metabolic activity towards acidic conditions. Unlike typical ion channels, proton channels remain open but diminish their activity depending upon internal pH. If large molecules such as morphine or naloxone blocked these channels, then the diminished efflux of H^+ would result in enhanced internal pH that would, by the time the planarian were tested after 2 h of exposure, resulted in diminished activity to minimize the accumulation.

We cannot identify with any assurance which receptor subtypes of morphine (or the μ receptor antagonist, naloxone) were associated with the two concentrations, 100 nM and 1 pM. The widening of the effectiveness of morphine around the 100 nM trough would suggest a μ receptor. The largest net change associated with co-exposure to the magnetic field and naloxone compared to naloxone only was also within the 100 nM range. However in this instance the combination enhanced, not attenuated, the decreased LMV values. If we assume that the presence of the magnetic field altered some aspect of receptor dynamics that widened the effective range of the dosage that produced the enhanced decrease of LMV values, then, it is possible that the naloxone was no longer a competitive antagonist but stimulated the receptor. If this argument is valid, then the 100 nM range effect could be related to the μ receptor.

The marked decrease in activity noted for the 100 nM and 1 pM ranges might also reflect another biochemical entity that is known to affect the behavior of planaria: melatonin. According to the review by Dubocovich et al. (2010) the two melatonin receptors, MT1 and MT2, are regulated by concentrations of melatonin from 30–400 pM and 1–1000 nM, respectively. The net difference in these two concentrations is within the same order of magnitude as the differences between the 100 nM and 1 pM separation measured in our study for the two peaks in minimum LMV values. Inward-rectified potassium channels are activated by melatonin and the best known signalling pathway for melatonin receptors is the inhibition of cAMP. Both processes could result in diminished activity. Itoh et al. (1999) measured melatonin levels of 0.4 pM per planarian during the dark phase and 0.1 pM during the light. As expected melatonin binding inhibits regeneration (Yoshizawa et al. 1991).

There are at least two possible mechanisms by which morphine effects could have been facilitated at 100 nM and particularly at 1 pM dosages. First, the disulfide bond between Cys113 and Cys190 residuals maintains the proper receptor confirmation for ligand binding (Dubocovich et al. 2010). A similar property is displayed by δ-opioid receptors. Morphine may have affected disulfide bounds in general and hence affected a receptor subtypes, including those specific to this opioid receptor subtype. Second, some melatonin agonists display a high affinity for MT1 and MT2 receptors but are antagonists for biogenic amine receptors such as 5-hydroxytryptamine (5-HT) serotonin. Additional research should reveal an underlying commonality between morphine receptor subtypes, melatonin receptor subtypes and the pivotal role of proton movements.

Conclusion

Quantitative measures of movements of flatworms can be sensitive indicators of concentrations of opioid-related compounds ranging from concentrations in the micromolar to picomolar range in the aqueous environment. General diminishment of movements occurred across all concentrations of morphine from micromolar to picomolar concentrations with particular enhancement in a narrow range of concentrations that are consistent with the two major receptor subtypes for opiates known to exist for planarian. Below picomolar concentrations the movement behavior did not differ from the vehicle (spring water), indicate there is a discrete threshold for the numbers of molecules required for this effect. Exposure to temporally-patterned magnetic fields known to produce analgesia in rodents also produced a generalized suppression of movement and appeared to widen the effective concentration rather than enhance the magnitude of the effect at the concentrations attributed to receptor subtypes.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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